Gibbes (H.) With the Author's Compliments.

On the Parasitic Nature of Cancer.

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ON THE PARASITIC NATURE OF CANCER.[1]

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In making an investigation into this subject it is necessary to take for comparison a condition where undoubted parasites produce changes in an organ or tissue which are equivalent to a new growth. For this purpose psorospermosis in the liver of the rabbit is generally admitted to be a typical condition, and a careful study of the changes found there, and the relation of the parasite to the newly formed tissue, should be fully determined in the first place, so that the process can be followed out in the cells of a growing carcinoma, where, from the arrangement of the tissues, this is infinitely more difficult.

On cutting into one of the small nodules found in the rabbit's liver, a substance can be expressed having a yellowish tint; this is found under the microscope to consist of large oval bodies, with others of smaller size varying in form, epithelium shed from the bile ducts, and leucocytes. On making sections through one of these nodules it is found to be composed of dilated bile ducts, the spaces in which are filled by masses of the material just described. The large oval bodies are seen, some free in the cavities, others wedged in between the epithelial cells lining them; the smaller, rounded forms occupy the same positions. It is evident that having proceeded so far, it is necessary to find some means by which these parasites in their various forms can be differentiated with absolute certainty from the cells of the bile ducts, for otherwise their relation to one another cannot possibly be determined.

This can only be done by double staining in such a manner that the parasites are colored distinctly from the cells of the part and their nuclei and this staining must be uniform throughout the section and not in patches, and every section must stain alike.

After trying a large number of coloring agents, I have succeeded in producing a double stain which will fulfil these conditions.

It is made as follows: Dissolve 2 c.c. of aniline oil in 10 c.c. of cologne or rectified spirit, make up to 100 c.c. with distilled water, and filter. This makes practically a saturated solution. With this aniline water make a 2 per cent. solution of rosanilin sulphate for stain No. 1, and a 1 per cent. solution of iodine (not methyl) green for solution No. 2.

[1] Read before the Association of American Physicians, at Washington, May, 1893.

To use the stain, filter No. 1 into a watch-glass, and place in it some sections of rabbit's liver which have been hardened in alcohol, and leave them in for ten minutes. Remove, and wash them in water to remove superfluous stain, then wash slightly in ordinary alcohol and place them in a watch-glass of filtered No. 2. It is better not to time them in the second stain, but to take them out and examine in distilled water, and when the original red color has become changed to a dull purple the staining will have proceeded far enough. Then wash thoroughly in distilled water until all excess of color has been removed, then wash in ordinary alcohol until no more color comes away, clear in oil of cloves, and mount in xylol balsam. On examining a section prepared by the above method, it will be seen at once that there is no difficulty in differentiating the parasites from the tissues affected by them.

APPEARANCES PRESENTED BY THE PARASITES.

1. Large oval cells, each with a capsule stained slightly a dull red, having inside a mass of material which has been stained a brilliant red.

2. Shrunken, wrinkled capsules, some empty, some with a small mass of granular material in them, which, however, has not taken the bright red stain. A few of these empty capsules show a number of sharply defined black granules.

3. Large oval cells without a capsule, having a granular appearance, the whole stained slightly a dull reddish color. In addition to these are cells of the same character, but round in outline. These are probably the large oval cells in transverse section. These cells vary. Some show a coarse granulation amounting to a network, while in others the granulation is very minute. Some also show a small round nucleus. Their size also varies, but the smallest is many times larger than any single columnar cell lining the bile ducts.

APPEARANCES PRESENTED BY THE ALTERED TISSUES.

A section through the dilated bile ducts shows a number of spaces or cysts lined with columnar epithelium, and into these spaces project papillomatous folds which branch and are also covered by columnar epithelium. These folds consist of fibrous trabeculæ, varying in thickness, having a homogeneous basement membrane on which the epithelium is situated. In the more delicate trabeculæ—that is, those most recently formed—the connective tissue is in process of development, and contains a large number of young connective-tissue corpuscles.

At the base of these folds this young connective tissue can be seen penetrating the liver substance and isolating the liver cells, so that in the substance of some of the folds one or more rows of liver cells are still left surrounded by connective tissue.

ACTION OF THE STAINS ON THE TISSUES.

The green stain has picked out all the nuclei by its action on the nuclear network, with the exception of the older connective-tissue corpuscles. By this stain the nuclei of the columnar cells covering the trabeculæ in folds are seen to be small, narrow, oval in shape, having a comparatively thick network; the nuclei of the liver cells at the base of the folds are much larger, round, or slightly oval, and have a finer network. The nuclei of the young connective-tissue corpuscles are of the usual character, and the leucocytes have taken the stain very deeply. These are the only elements stained by the green.

The fibrous tissue and protoplasm of the liver cells are stained a purplish red, and the protoplasm of the columnar cells has taken the same tinge, the color being darker in the liver cells than in the other two.

THE RELATION OF THE PARASITES TO THE ALTERED TISSUE.

Taking a section which has been cut as thin as possible, and in which the different elements have been differentiated as described above, we find, in the first place, a mass of parasites filling up the spaces between the folds, a number of them being a bright red, the others having only a faint tinge of dull red; amongst these are a number of desquamated columnar cells in various stages of disintegration; their nuclear network, however, is stained brightly with the green. There are also a number of deeply stained small round cells (green) which appear to be leucocytes. On examining the epithelium, we find wedged in between the cells a number of the parasites, some in the capsulated form a brilliant red, others a dull red, with no capsule. They are plainly wedged in between the cells, and reach down to the basement membrane. It is, however, a difficult matter to get at the exact relationship of one to the other in the section of the fully formed fold. But if we look for a small bile duct in which the process of dilatation is only just commencing, it becomes an easy matter to understand it. There are a number of small bile ducts to be found at the base of the folds which contain several parasites, and in proportion to the number of these has the process of dilatation advanced. In the smallest a few parasites are seen, one or two adult forms, while there may be five or six that have not developed a capsule; the majority, however, are free in the lumen of the duct, a few are wedged in between the cells. In some ducts the process has gone further, and small folds are just beginning to form, and in these ducts the lumen is much larger and the parasites more numerous. The relation of the parasites is, however, very plain, and by using a binocular microscope with a high power it can be ascertained beyond a doubt; the parasites that are wedged in between the columnar cells by their growth and presence cause desquamation and disintegration of contiguous cells, but in no case

are they contained in the columnar cells. This would be a physical impossibility, as the smallest forms of the parasites are much larger than the columnar cells. It is, of course, possible for the parasite, in an early stage of its growth, to get inside a cell and expand it as it grows. I cannot, however, find any parasite small enough to do this, neither do any of the columnar cells show evidence of any displacement of their nuclei, which must take place if another body was growing inside them.

On examining some of the large cells that have no capsule, a few will be found which have had a portion cut off in making the section. These cells show a margin of granules, while the centre is free from them. This shows that the granules are on the outer margin of the cells, and the binocular microscope confirms this view. We have, then, a large oval cell having a granular covering. On examining a number of these, it is at once apparent that this granular covering varies very much in different cells. In some it is very fine and difficult to make out, having scarcely any appreciable thickness, in others the granules are much larger and the coating is thicker. Further examination shows that this granular covering increases in thickness and in the size of its elements until definite patches are seen covering the cell. In some this change has gone on until the cell is almost covered; this is the development of the capsule, and the appearances described might easily be considered as the expansion of one cell by the growth of another inside it. All these stages in the development of the capsule can be seen going on in the spaces of the dilated bile ducts. It seems to me that this explains the view held by some that the parasite inhabits the cells of the bile ducts. If we consider the enormous number of parasites that are found free in the spaces of the dilated bile ducts, and the very small number that are found wedged in between the cells, also that every stage in development is going on in those lying free, and also take into consideration the fact that the smallest form of the parasite that we can detect is very much larger than the columnar cell of the bile duct, it seems to me that we must accept as a fact that the action of the parasites is simply one of chronic irritation caused by their rapid multiplication in the lumen of these vessels. This is a very important consideration, as we do not know how far, in many cases, new growths are produced simply by chronic irritation. I have seen several cases where horny growths which had existed for many years were converted into epitheliomata by the action of irritants. In another case, where lycopodium had been used as a dressing, I found the seeds deeply imbedded in a new growth. They may, of course, have had nothing to do with the formation of the neoplasm. But in a case of villous growth in the bladder removed by Sir Henry Thompson, I found a wheat-straw imbedded in its centre. In this case I have no doubt the new growth was caused by the foreign body.

The next question to be considered is the cancer parasite and its

relation to the cancer cell. On reading the recent literature on this subject, the first thing that strikes one is the diversity of opinion that exists as to the appearances presented by the parasite and its frequency, some observers finding it in every case in such numbers that it could be estimated by weight, while others found it in only about 4 per cent. of the cases examined. From the illustrations given, it would seem that all the observers were not looking at the same object. Some give special methods of hardening the tissue, while others found the parasite in material hardened in the usual manner. To test the value of these special methods, and find out what their action would be on tissues, I hardened portions of a fresh cancer by the methods used in my laboratory, and also by bichloride of mercury, osmic acid and Müller's fluid, and some other methods described by different observers; at the same time I hardened portions of normal ox liver by all these processes. Sections were made of all the material and stained with logwood, and also with the special stain I have already described. The sections of cancer and liver hardened in the usual manner showed the normal liver as it is known to be, while there was nothing to be seen in the sections of cancer which could be considered parasitic; but in the sections hardened in bichloride of mercury and in osmic acid and Müller's fluid, both liver and cancer were so much altered by the hardening process that it was not difficult to realize how the characteristic organism of cancer could be found and estimated by weight. Those observers, however, who have only found the so-called parasite in a small percentage of the cases examined, have not been describing effects produced by bad hardening; the appearances they have figured I have long been familiar with. They are, I think, capable of a different explanation than that given. I have gone over the large collection of cancer specimens I have made in the last fourteen years, and I find these appearances in only a few of them. In 1879, while working at karyokinesis, I obtained a specimen of cancer in the fresh condition, which, after hardening, showed in the section a number of very large cells. In these I found mitotic division most beautifully shown, but the process was going on inside the large cell; the original cell was enormously enlarged, its nucleus and some of its protoplasm pushed to one side, while in the centre was a small mass of protoplasm containing a nucleus. This nucleus, in various cells, showed all the stages of mitotic division, from the simple aster to two fully formed nuclei. I considered this to be a typical example of the endogenous division of cells. In 1886 I photographed this specimen amongst a number of others, showing various conditions in cancer development, and in 1891 I used this as an illustration of endogenous division in my work on *Practical Pathology and Morbid Histology*. I have only a few cases in which this process appears.

For the purpose of studying the parasitic nature of cancer I have used

cases where the development of the cells can be observed, such as in the axillary glands, where the process is secondary to carcinoma mammæ, and where the operation was performed in an early stage of the disease, and also in cases where recurrence had taken place and the growth had been removed twice or three times. In recurrent cancer of the breast small nodules are found in the muscles, and their development and relation to the muscular tissue is very interesting. Examining that portion of a section which shows the cancer cells invading the muscles, changes in the muscular fibres which are at some little distance from the cancer cells are at once apparent. Some of the fibres show spaces in their substance somewhat similar to the appearances found in striped muscle in cases of myxœdema; in others, the muscle substance is completely broken up and the muscle corpuscle is seen as a shrunken nucleus surrounded by an empty space. All these changes take place before a single cancer cell is in apposition with the muscle fibre.

The next change is the appearance of one or two cancer cells in the centre of the broken-down muscle fibre. These increase until the fibre is replaced by cancer cells and the sarcolemma is either changed into fibrous tissue or is replaced by it. The method by which the cancer cells gain access to the centre of the muscle fibre is not apparent, and requires further investigation. In all these cases active growth of the cancer elements can be accurately observed, and I hold that where a pathological process is in an active stage of development, there the cause, if capable of demonstration, should be found. I considered that in these cases I ought to find the parasite, if the disease process were caused by one. The most careful examination of a number of cases has failed to show anything that could be construed into any form of parasite —that is, in those sections hardened by a method which gave normal appearances in sections of normal liver.

When we consider the many different forms of cancer, it does not eem possible that they can be the result of the action of any one form of parasite; and, further, when we study the differences between two forms, such as squamous epithelioma and glandular carcinoma, we find the differences so marked that, although they have certain points in common, yet they are practically different diseases. In squamous epithelioma we have a condition in which the epithelium receives some impetus at a certain point which causes it to grow downward into the tissue below, and there to branch out in every direction. It is at the one point, however, that the growth takes place, and the newly formed epithelial tissue will grow sideways under the normal epidermis without having any effect on it. It has also no effect upon the fibrous connective tissue into which it grows, as a rule. I have, however, seen an enormous hyperplasia of yellow elastic tissue produced by the growth of an epithelioma in the eyelid.

On the other hand, we have a glandular carcinoma, starting in secretory epithelium, and consisting of cells derived from this epithelium, and in every case this new growth affects the fibrous connective tissue in such a manner that a supporting framework is formed for it as it grows, and in this are contained new bloodvessels for its nourishment. Although these two forms differ so much in their construction, there is one point of absolute resemblance between them. A cell from either form, on being carried to a distant organ, will at once reproduce a cancer of the same type as that from which it started. It therefore follows that if a parasite is required, it must be the cell itself; but that gives no explanation as to how the first cancer cell became cancerous.

Sarcoma must be considered on an entirely different basis. Where I have been enabled to examine a sarcoma in its earliest condition, that is, where a young growth is starting in connection with an established sarcomatous condition, I have found that it always consisted of embryonic connective tissue, and, according to my observations, all mesoblastic neoplasms begin in this way. Whether they receive the impetus which decides that they are to become sarcomata or to remain benign at the commencement or later in their growth, would seem to vary in different cases.

Conclusions.—1. The dilatation of the bile ducts in the liver of the rabbit is caused by the chronic irritation produced by the multiplication of the coccidium oviforme (Leuckart) in them, and the appearances which have given rise to the view that these parasites are developed in the columnar cells of the bile ducts are caused by the different stages in the development of the capsule.

2. The appearances found in a small percentage of glandular carcinomata are caused by endogenous cell-formation; the large majority of glandular carcinoma show nothing which can be considered parasitic when hardened by any method which, when applied to normal tissue, will give a typical normal section.